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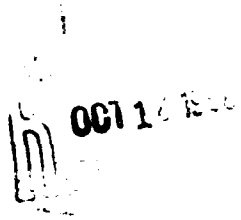

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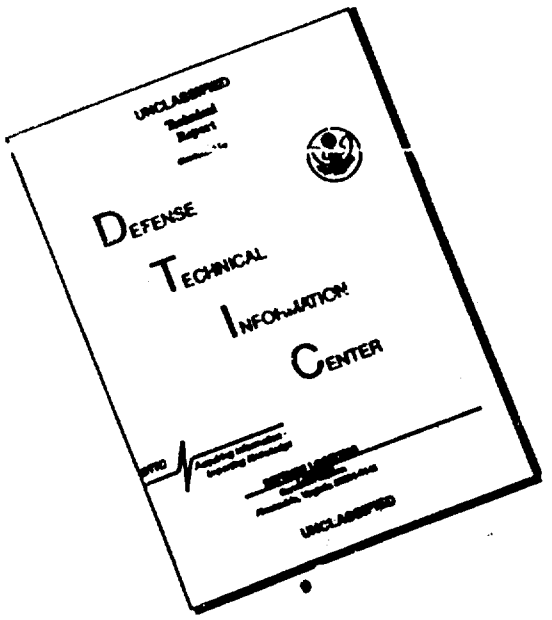

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RESULTS OF AND PROSPECTS FOR THE STUDY AND USE OF LIVE TULAREMIA VACCINE IN THE USSR

N. G. Olsuf'yev

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Tularemia was diagnosed for the first time in the USSR in 1926. Within a few years of its discovery tularemia attracted the attention of medical workers because of widespread epidemic outbreaks of the disease in several regions of the European and Asiatic USSR. Hundreds and thousands of persons contracted the disease. There were many cases during World War II, chiefly in the front areas and on territories temporarily occupied by the German invaders. The increased incidence resulted from the enormous multiplication of mouselike rodents caused by the chaotic management of agriculture, reduction of the sown areas, poor harvesting of the crops, etc.

The many outbreaks of tularemia, its prolonged course, and vastness of its natural foci demanded that efforts be made to try to prevent it. Owing to the difficulty of preventing the disease by exterminating the rodents, the sources of the infection, or by altering the routes of transmission of the causative agent, investigators concentrated on the development of vaccines.

The first to be studied were killed vaccines. In 1939 L. M. Khatenever and G. Ya. Sinay in southeastern Kazakhstan (Ush-Tobe station) inoculated 41 persons subcutaneously and in part intracutaneously with glycerinated heat-killed tularemia vaccine. This was the first time in history that people had been vaccinated against tularemia. It was preceded by the authors' experiments on laboratory animals. The vaccine was inoculated three times and twice. The

vacc'nates' blood was found to contain antibodies to the tularemia microbe, but the prophylactic value of the vaccine was uncertain.

Further study of killed tularemia vaccines in the USSR and abroad did not justify the hopes placed in them, for tests on animals and humans showed that they were not too effective. This led investigators to look for a live tularemia vaccine. B. Ya. El'bert and N. A. Gayskiy were pioneers in this field. Their thorough investigation of tularemia and the mechanisms of immunity as well as of the variability of the tularemia microbe culminated in 1934-1936 in the preparation of the Moskva vaccinal strain and successful tests on 34 volunteers. The vaccine was readily tolerated when inoculated subcutaneously and a single inoculation produced a high degree of immunity (N. G. Olsuf'yev et al. 1962). At the same time (1936) El'bert was able to achieve effective immunity in laboratory animals by epicutaneous application (scarification) of live tularemia vaccine.

After further research on the vaccine, Gayskiy devised a technique for attenuating the tularemia microbe on synthetic media and obtained two vaccinal strains: No. 15 and Ondata IV. Both strains were carefully tested on laboratory animals and in 1942 successfully administered to 50 volunteers. By 1943 some 5614 persons living in tularemia foci were inoculated subcutaneously under his supervision. Strain 15 was found to confer greater immunity and it was subsequently used for the manufacture of vaccine. The vaccine was first prepared as a suspension in physiologic solution, but later on Gayskiy together with Ye. M. Golinevich used vacuum drying to produce a more stable preparation. M. M. Faybich and T. S. Tamarina in 1944 continued to perfect the desiccation method until the vaccine was ready for mass production. In 1945 El'bert et al. tested his proposed epicutaneous method on humans, having prepared the vaccine from Gayskiy's strain 15 on M. S. Drozhevkina's liquid egg-yolk medium. This vaccine was successfully administered at the end of 1945 to many people during an outbreak of tularemia. From the 12th day on none of the vaccinates came down with the disease.

Thus, by the end of 1945 the production and large-scale testing of live tularemia vaccine was largely completed. In 1946 El'bert and Gayskiy were awarded the State Prize for their achievement. In the same year the vaccine was administered to 468,000 persons in several regions of the USSR. The numbers vaccinated increased greatly in the years thereafter.

During the past 20 years, besides the widespread use of live tularemia vaccine in the USSR, comprehensive studies have been made

on its preventive value under a variety of epidemiological conditions and on improved methods of preparing and administering it. Also, extensive microbiological and immunological research has made important contributions to the theory and practice of vaccinoprophylaxis of tularemia. The following took part in this work: I. S. Tinker, O. S. Yemel'yanova, I. N. Mayskiy, A. V. Mashkov, V. A. Yudenich, V. S. Sil'chenko, M. F. Shmuter, M. S. Drozhevkina, T. I. Puchkova, Me. Ye. Kal'k, Z. D. Khakhina, N. A. Kazberyuk, S. P. Karpov, Yu. A. Myasnikov, G. P. Uglovoy, R. A. Savel'yeva, V. P. Borodin, M. A. Aykimbayev, V. G. Pilipenko, N. D. Altareva, P. N. Burgasov, N. K. Vereninova, M. M. Kirvel', L. S. Kolyaditskaya, I. M. Martinevskiy, L. S. Matveyets, A. D. Zlatkovskiy, V. P. Motornya, R. A. Saltykov, A. A. Selezneva, I. A. Chalisova, and others. We shall now discuss the major results of their studies.

The theoretical studies fully confirmed El'bert and Gay'skiy's concept that vaccinal cultures occupy an intermediate position between the original virulent S-form and the avirulent and nonimmunogenic R-variant obtained on synthetic nutrient media. Also confirmed were their data on the presence of two antigenic complexes in the tularemia microbe and the connection of the virulent and immunogenic properties only with one of the antigenic complexes lost in the course of attenuation. The latter complex is bound with the membrane substance of the cell and is analogous to the Vi- (or K-) antigenic substances of other gram-negative bacteria (I. S. Tinker, 1951; N. G. Olsuf'yev, and O. S. Yemel'yanova, 1957). O-substances represent the other complex. The vaccinal cells, designated as the S-R-variant, contain O-substances and less Vi-substances than do the virulent strains. The residual virulence and immunogenicity of the vaccinal strains is related to the Vi-substances. Chemical investigations showed that in the process of conversion to the R-form the antigenic substances of the tularemia bacteria lose their lipids while their proteins become partly depleted (G. K. Shipitsina and O. S. Yemel'yanova, 1956).

The hereditary properties of the tularemia vaccinal strains are only relatively fixed. By repeatedly passaging a vaccinal culture on synthetic nutrient media one can convert it into the avirulent and nonimmunogenic R-form and, contrariwise, by repeatedly passaging a vaccinal culture in a susceptible animal one can make it approximate the S-form in virulence (L. A. Pomanskaya and Yu. A. Myasnikov, 1955; O. S. Yemel'yanova, 1960). However, there is no danger of the vaccinal strains again becoming virulent when inoculated into human beings because there is only a single passage of the vaccinal culture through the human organism as a result of vaccination.

The antigenic substances of the virulent S-cell extracted by Bouavain's, Larson's, or other methods confer only partial immunity on laboratory animals, an indication of the lability of the \i-substances.

The techniques of attenuating tularemia bacteria proposed by Gaysk.v were tested and slightly refined by Yemel'yanova and Mayskiy. They found that any virulent strain can be attenuated, but at different rates, depending on the method used and the individual properties of the strain. Yemel'yanova prepared a new vaccinal strain 155, which was then used with strain 15 to manufacture vaccine.

Study of the variability of tularemia bacteria was greatly facilitated by the use of Yemel'yanova's nutrient media (blood-fish-yeast agar with cystine and glucose). The isolated colonies (differing in structure in relation to the degree of attenuation) that were obtained on this medium made it possible to study in detail the dissociation of cultures. They also afforded new opportunities for checking and perfecting the vaccine.

Even storage of the vaccinal strains in a dry (lyophilized) state with occasional transfers does not guarantee against further attenuation. The valuable immunogenic properties can be restored only by the "animalization" method, i.e., by passages through laboratory animals (V. M. Motornaya, 1953; O. S. Yemel'yanova, 1960). Gayskiy's strain, which has been used for many years to produce vaccine, was twice restored in this way -- in 1954 and in 1962 (O. S. Yemel'yanova).

The method of manufacturing the vaccine has been greatly improved. The highly efficient deep method of growing vaccinal cultures has been introduced. Nutrient media which reduce the dissociation of bacteria and detachment of nonimmunogenic cells to a minimum are used at various stages (L. S. Kolyaditskaya). More accurate dosage prevents some series of the vaccine from provoking excessive reactions. New methods of quality control have been developed (O. S. Yemel'yanova). Numerous trials on human beings showed that the vaccine is quite stable and that the scarification technique, if properly employed, ensures a take in 96 to 98% of the cases, sometimes in almost 100%. In 97% of the vaccinates (with positive results) the skin reaction appears within 5 days and disappears between the 20th and 30th days. No more than 3.5 to 4.4% of the individuals have systemic reactions such as malaise, enlarged lymph nodes, etc. (N. G. Olsuf'yev et al., 1958; A. N. Gudoshnik et al., 1965). Allergic rashes are extremely rare (M. F. Shmuter, 1953).

During the first few years of mass use of the vaccine, the vaccine took in some 85% of the cases and provoked side effects in 25 to 30% owing to the faulty method of production (liquid egg-yolk vaccine) and poor quality control.

The peculiarities of immunogenesis were also studied in detail. Experiments on laboratory animals confirmed Gayskiy's view that the process caused by the vaccine is not regional. The vaccinal cells penetrate into the viscera and at the multiplication sites they give rise to a specific granulomatous process in the tissues. But unlike the acute infection produced by a virulent culture, the vaccinal process is benign. There is only a slight insemination of the organs and tissues with the vaccinal cells, and the resulting proliferative changes in the tissues do not lead to the necrosis characteristic of the acute infection (I. A. Chalisov and M. G. Spasskaya, 1946; A. V. Mashkov, 1952; others). Numerous animal experiments and tests on humans revealed that the tularemia vaccine elicits positive, strictly specific serological and allergic reactions. However, antibody formation and sensitization are less intense than in a regular case of the disease. Allergic sensitivity can be detected by intracutaneous, inhalation or other methods of introducing the antigen into the body. An allergic reaction of the slow type develops in response to intracutaneous injection of corpuscular antigen, but some antigenic substances of the tularemia microbe can cause an accelerated type of reaction in man (the "tuallergen" of I. N. Mayskiy and G. K. Shipitsina, 1952).

Other studies confirmed the view of El'bert and Gayskiy that the resistance of the immune organism is related to its specific allergic reconstruction aimed at rapid elimination of the causative agent from the focus (I. N. Mayskiy, 1953; T. A. Kalitina, 1953).

The mechanisms of immunity have been little studied. It has been said (B. Ya. El'bert and N. A. Gayskiy, 1941) that a nonsterile immunity is formed in tularemia, but control experiments have not verified this (L. M. Khatenever, 1946; others). The basis of immunity to tularemia seems to be the same as in other bacterial infections, i.e., specific mobilization of the natural defenses (inflammation, phagocytosis) under the influence of the antibodies formed, with the cellular elements of the macroorganism playing a major role. Tissue culture studies have shown that the phagocytes of vaccinated animals are more capable of digesting (lysing) virulent tularemia bacteria than are the phagocytes of nonvaccinated animals (Thorpe and Marcus, 1964).

In the presence of acquired immunity of sufficient strength, small numbers of virulent bacteria penetrating into the body are

usually arrested at the tissue barrier of the skin and regional lymph nodes. In the case of large doses of the causative agent, a few may reach the viscera (R. A. Savel'yeva and A. P. Gindin, 1965).

The use of diagnostic immunological reactions, especially the tularin test, is very valuable in determining the duration of immunity in vaccinates, the desirable times of revaccination, and the immunological structure ("immune layer") of the population. El'bert and Gayskiy were the first to point out the value of the tularin test in detecting immunity. Tularin is now applied epicutaneously, a procedure that prevents undue local and sometimes systemic reactions associated with the intracutaneous mode of inoculation. To facilitate the production of tularin, it has been suggested that the vaccinal strain be used instead of the virulent one (N. G. Olsuf'yev).

Many studies demonstrated the value of the agglutination test in determining immunity after tularemia vaccination. The results of the serological and allergic reactions were usually found to coincide (M. F. Shmuter, 1953; V. A. Yudenich, 1954; others). However, at longer intervals after vaccination negative agglutination reactions have sometimes occurred despite positive allergic reactions. In such cases antibodies can be detected by the more sensitive passive hemagglutination test that was developed just a few years ago (I. S. Meshcheryakova, 1964).

When the tularemia vaccinations were properly carried out, the allergic skin reaction, a sign of immunity, occurred within a year in 92 to 98% of the vaccinates, within 5 to 6 years in 75 or 90%, within 8 years in 58 or 83% (V. S. Sil'chenko, 1960), and within 15 years in 21% (G. P. Uglovoy, 1966). There are some data indicating that immunological reconstruction is less stable in children from 7 to 14 years of age after vaccination (V. S. Sil'chenko, 1953; G. P. Uglovoy, 1953; others).

Animal experiments have demonstrated the possibility of administering the vaccine by the aspiration, nasal, and alimentary routes. However, the level of immunity thus achieved is no higher than with subcutaneous or intracutaneous inoculation (M. M. Kirvel', 1953; I. F. Mikhaylov and R. A. Savel'yeva, 1952). In tests on humans (N. I. Aleksandrov and N. Ye. Gefen, 1962), vaccination by the aspiration method ensured a satisfactory development of immunity. An obstacle to the widespread use of the technique is the need to screen out individuals immune to tularemia, for they may have an allergic reaction to intranasal administration of the vaccine with involvement of the respiratory tract.

The use of associated vaccines, or simultaneous inoculation of several vaccines, is an important development of the past 10 years. On the basis of his experiments with simultaneous epicutaneous vaccination of guinea pigs against tularemia and smallpox, El'bert was the first to mention the possibility of using associated live vaccines (including tularemia vaccine). Later tests on animals and humans showed that live tularemia vaccine can be used with live brucellosis vaccine (V. G. Pilipenko and A. M. Polyakova, 1955; Ye. A. Gubina and G. P. Uglovoy, 1958; others) or with live brucellosis and plague vaccines (N. K. Vareninova et al., 1958; V. G. Pilipenko, et al., 1959; others) without interfering perceptibly with the building of immunity to either component. Other experiments showed that live tularemia vaccine can be used with BCG (R. A. Savel'yeva, 1964) or live yellow fever vaccine (S. A. Ananyan and G. I. Medvedeva, 1959) or combined with killed virus encephalitis vaccines (N. V. Ryzhov, 1962) and others.

Analysis of the results of revaccination showed that in individuals with pronounced postvaccinal immunity, the reaction develops very quickly with increase in the immunological indices (L. S. Matveyets, 1960) and lengthening of the period of immunity. In individuals who have lost their immunity completely, revaccination restores it to the level following the initial vaccination and the vaccinal process takes place at the usual time and with the same intensity. Following a study of extensive data on the time required for immunity to disappear in those given tularemia vaccine, the time of revaccination has been officially set at five years after the initial vaccination. The technique of inoculation is the same.

Soviet investigators have carefully studied the prophylactic value of El'bert-Gayskiy vaccine. Observations in various foci, including very active ones, invariably confirmed the fact that vaccinates are protected against infection through the skin, lungs, alimentary tract, etc. The overwhelming majority of vaccinates did not get the disease for several years, whereas cases were recorded among nonvaccinates exposed to the same epidemiological conditions (V. S. Sil'chenko, 1953, 1960; V. A. Yudenich, 1953; N. G. Olsuf'yev, 1953; others).

Thanks to the prolonged immunity enjoyed by most vaccinates, systematic vaccination campaigns in enzootic areas are fully justified even in the absence of urgent epidemic indications (V. S. Sil'chenko).

As mentioned above, tularemia was particularly prevalent in the Soviet Union during World War II, especially in 1945. The sick

rate remained fairly high from 1946 to 1949. During this time live tularemia vaccine was undergoing trials, first in a few regions, then, in 1949, in many areas with natural foci of the disease. Some 14 million persons were vaccinated in 1949 as compared with 5 million during the preceding three years. Thereafter some 7 to 14 persons were vaccinated every year. All rural residents 7 years and older living in the natural foci of tularemia and children from 2 years on in especially active foci were vaccinated. Also vaccinated were city people traveling to such foci. The prophylactic value of the inoculations was not slow in manifesting itself. Beginning in 1950 the incidence declined sharply and has been continuing to do so with minor fluctuations ever since. During the past 6 years the incidence was much lower than during the first 5 years after the war, reaching the lowest point in 1965, when it was 1000% lower than in 1945. There have been mostly sporadic cases, with minor outbreaks extremely rare. This has happened despite the epizootics among rodents that take place every year in various republics or oblasts. In recent years some 400 to 500 cultures of the causative agent of tularemia have been isolated annually (incomplete data) from rodents, ticks, water, etc. The successful efforts to reduce the tularemia rate is largely due to massive vaccination of the population, embracing some 60 million persons living in natural foci of tularemia or traveling there from cities.

The tularin test has played a major role in determining the immunological structure of the population ("immune layer") created by the vaccinations. It is given randomly every year to at least 400,000 to 500,000 persons living in the areas where vaccination campaigns have been conducted. Experience has shown that at least 90% of the population in active tularemia foci must be immune (as determined by the tularin test) for the area to be safe from epidemics.

In addition to vaccination, other measures contributed to the decreased incidence of the disease. For example, in tularemia foci of the meadow-field and steppe types, better management of agriculture, enlargement of the plowed area, prompt mechanized harvesting of the crops, plowing again in the fall, etc. were helpful in limiting the multiplication of rodents. In some places campaigns to exterminate rodents and ticks and sanitary protection of sources of water, warehouses, etc. were other useful preventive measures.

The successful control of tularemia achieved in the Soviet Union must be reinforced in the coming years by practical medical workers and by researchers. One of the main tasks is to elucidate

the geography of the natural foci of tularemia, a prerequisite of total eradication of the disease. In the established foci, careful vaccination and revaccination of the population along with other steps should be continued. There should be further study of vaccinoprophylaxis as a means of eradication. Special attention ought to be paid to preserving in the laboratory the valuable properties of the vaccinal strain now used and to obtaining new strains. The methods of manufacturing the vaccine should be improved. More research is needed on the immunology of tularemia, especially on the mechanisms of immunity. Finally, additional efforts should be made to clarify the conditions of existence of the natural foci and to devise methods for improving them. All this will lead to the total eradication of tularemia in the Soviet Union.